

Homology Comparison of Pitilosarcus Gurneyi and Renilla Muleri
GFPs with Aequorea Victoria wt and Enhanced GFPs

Pitilosarcus	MNRNVLKNTGLKEIMSAKASVEGIVNNHVFSMEGFGKGNVLFGNQLMQIR
Renilla	MSKQILKNTCLQEVMSYKVNLEGIVNNHVFTMEGCGKGNILFGNQLVQIR
EGFP (Aequorea)	MSKGEELFTGVVPIL---VELDGDVNGHKFSVSGEGEGDATYGKLTCLKFI
Aequorea	MSKGEELFTGVVPIL---VELDGDVNGHKFSVSGEGEGDATYGKLTCLKFI
	*.: * : : . : : * * * * * : : * * : : : :
Prim. consensus	MSKGE222TG2V2I2S2KVEL2G2VN2H2FS22GEG2G2A22G222L222
Pitilosarcus	VTKGGPLPFAFDIVSIAFQYGNRTFTKYPDDIA--DYFVQSFPAFFFYER
Renilla	VTKGAPLPFAFDIVSPAFOYGNRTFTKYPNDIS--DYFIQSFPAFFMYER
EGFP	CTTG-KLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQER
Aequorea	CTTG-KLPVPWPTLVTTFSYGVQCFSTRYPDHMKQHDFFKSAMPEGYVQER
	*. * * * . : : : * * : * : * * : : * : * . : : * * *
Prim. cons.	2T2G22LP2222222T2FQYQ222F22YPD22KQHD2FK222P2G2V2ER
	Chromophore
Pitilosarcus	NLRFEDGAIVDIRSDISLEDDKFHYKVEYRGNGFSPNGPVMQKAILGMEP
Renilla	TLRYEDGGLVEIRSDINLIEDKFVYRVEYKGSNFPDDGPVMQKTIILGIEP
EGFP	TIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN
Aequorea	TIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN
	. : : * . . . * : : : * . : : * : * . * . : : :
Prim. cons.	T22F2D2GN2K2R222K2EGD22V2R2E2KGIDF2EDG22222K222N22
Pitilosarcus	SFEVVMYMN----SGVLVGEVDLVYKLESGNYYSCHMKTfYRSKGGVKEFP
Renilla	SFEAMYMN----NGVLVGEVILVYKLNSGKYYSCHMKTLMKSKGVVKEFP
EGFP	SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLP
Aequorea	SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLP
	* . . . * . * : * . : . . : * : . : *
Prim. cons.	S22VY2M2DKQKNG22V222I22222D22V2222H222NTP222G2222P
Pitilosarcus	EYHFIHHRLEKTYVEEGSFVEQHETAIAQLTTIGKPLGSLHEWV
Renilla	SYHFIQHRLEKTYVEDGGFVEQHETAIAQMTSIGKPLGSLHEWV
EGFP	DNHYLS--TQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYK
Aequorea	DNHYLS--TQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYK
	. * : : . : : : : . : * . : : * : * . * . : :
Prim. cons.	D2H22SHR222222D2N2222H22222VTA2G22LG222222

Alignment data :

Pitilosarcus G. vs. Aequorea

Identity (*) : 55 is 22.45 %
Strongly similar (:) : 61 is 24.90 %
Weakly similar (.) : 35 is 14.29 %
Different : 94 is 38.37 %\

Renilla M. Vs. Aequorea

Identity (*) : 60 is 24.39 %
Strongly similar (:) : 65 is 26.42 %
Weakly similar (.) : 28 is 11.38 %
Different : 93 is 37.80 %

Renilla vs. Pitilosarcus

Alignment length : 238
Identity (*) : 184 is 77.31 %
Strongly similar (:) : 31 is 13.03 %
Weakly similar (.) : 14 is 5.88 %
Different : 9 is 3.78 %

Beau Peelle 10/15/99

Figure 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGTTATACAC	AAGTGTATCG	CGTATCTGCA	GACGCATCTA	GTGGGATTAT	TCGAGCGGTA	60
GTATTTACGT	CAGACCTGTC	TAATCGAAAC	CACAACAAAC	TCTTAAATA	AGCCACATTT	120
ACATAATATC	TAAGAGACGC	CTCATTTAAG	AGTAGTAAAA	ATATAATATA	TGATAGAGTA	180
TACAACCTCTC	GCCTTAGACA	GACAGTGTGC	AACAGAGTAA	CTCTTGTTAA	TGCAATCGAA	240
AGCGTCAAGA	GAGATAAG	ATG AGT AAA	CAA ATA TTG	AAG AAC ACT	TGT TTA	291
	Met Ser Lys	Gln Ile Leu	Lys Asn Thr	Cys Leu		
	1	5		10		
CAA GAA GTA	ATG TCG TAT	AAA GTA AAT	CTG GAA GGA	ATT GTA AAC	AAC AAC	339
Gln Glu Val	Met Ser Tyr	Lys Val Asn	Leu Glu Gly	Ile Val Asn	Asn Asn	
	15	20		25		
CAT GTT TTT	ACA ATG GAG	GGT TGC GGC	AAA GGG AAT	ATT TTA TTC	GGC	387
His Val Phe	Thr Met Glu	Gly Cys Gly	Lys Gly Asn	Ile Leu Phe	Gly	
	30	35		40		
AAT CAA CTG	GTT CAG ATT	CGT GTC ACG	AAA GGG GCC	CCA CTG CCT	TTT	435
Asn Gln Leu	Val Gln Ile	Arg Val Thr	Lys Gly Ala	Pro Leu Pro	Phe	
	45	50		55		
GCA TTT GAT	ATT GTG TCA	CCA GCT TTT	CAA TAT GGC	AAC CGT ACT	TTC	483
Ala Phe Asp	Ile Val Ser	Pro Ala Phe	Gln Tyr Gly	Asn Arg Thr	Phe	
	60	65		70	75	
ACG AAA TAT	CCG AAT GAT	ATA TCA GAT	TAT TTT ATA	CAA TCA TTT	CCA	531
Thr Lys Tyr	Pro Asn Asp	Ile Ser Asp	Tyr Phe Ile	Gln Ser Phe	Pro	
	80	85		90		
GCA GGA TTT	ATG TAT GAA	CGA ACA TTA	CGT TAC GAA	GAT GGC GGA	CTT	579
Ala Gly Phe	Met Tyr Glu	Arg Thr Leu	Arg Tyr Glu	Asp Gly Gly	Leu	
	95	100		105		
GTT GAA ATT	CGT TCA GAT	ATA AAT TTA	ATA GAA GAC	AAG TTC GTC	TAC	627
Val Glu Ile	Arg Ser Asp	Ile Asn Leu	Ile Glu Asp	Lys Phe Val	Tyr	
	110	115	120			
AGA GTG GAA	TAC AAA GGT	AGT AAC TTC	CCA GAT GAT	GGT CCC GTC	ATG	675
Arg Val Glu	Tyr Lys Gly	Ser Asn Phe	Pro Asp Asp	Gly Pro Val	Met	
	125	130		135		
CAG AAG ACT	ATC TTA GGA	ATA GAG CCT	TCA TTT GAA	GCC ATG TAC	ATG	723
Gln Lys Thr	Ile Leu Gly	Ile Glu Pro	Ser Phe Glu	Ala Met Tyr	Met	
	140	145		150	155	
AAT AAT GGC	GTC TTG GTC	GGC GAA GTA	ATT CTT GTC	TAT AAA CTA	AAC	771
Asn Asn Gly	Val Leu Val	Gly Glu Val	Ile Leu Val	Tyr Lys Leu	Asn	
	160		165		170	
TCT GGG AAA	TAT TAT TCA	TGT CAC ATG	AAA ACA TTA	ATG AAG TCG	AAA	819
Ser Gly Lys	Tyr Tyr Ser	Cys His Met	Lys Thr Leu	Met Lys Ser	Lys	
	175	180		185		
GGT GTA GTA	AAG GAG TTT	CCT TCG TAT	CAT TTT ATT	CAA CAT CGT	TTG	867
Gly Val Val	Lys Glu Phe	Pro Ser Tyr	His Phe Ile	Gln His Arg	Leu	
	190	195		200		
GAA AAG ACT	TAC GTA GAA	GAC GGG GGG	TTC GTT GAA	CAG CAT GAG	ACT	915
Glu Lys Thr	Tyr Val Glu	Asp Gly Gly	Phe Val Glu	Gln His Glu	Thr	
	205	210		215		
GCT ATT GCT	CAA ATG ACA	TCT ATA GGA	AAA CCA CTA	GGA TCC TTA	CAC	963
Ala Ile Ala	Gln Met Thr	Ser Ile Gly	Lys Pro Leu	Gly Ser Leu	His	
	220	225		230	235	
GAA TGG GTT	TAA ACACAGTTAC	ATTACTTTTTT	CCAATTCGTG	TTTCATGTCA	AATAAT	1021
Glu Trp Val	*					
AATTTTTTAA	ACAATTATCA	ATGTTTTGTG	ATATGTTTGT	AAAAAAAAAA	AAAAAAAA	1079

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCGGCACGAG	CTGGCCTCCA	CACTTTAGAC	AAA	ATG	AAC	CGC	AAC	GTA	TTA	AAG	54					
				Met	Asn	Arg	Asn	Val	Leu	Lys						
				1				5								
AAC	ACT	GGA	CTG	AAA	GAG	ATT	ATG	TCG	GCA	AAA	GCT	AGC	GTT	GAA	GGA	102
Asn	Thr	Gly	Leu	Lys	Glu	Ile	Met	Ser	Ala	Lys	Ala	Ser	Val	Glu	Gly	
		10					15					20				
ATC	GTG	AAC	AAT	CAC	GTT	TTT	TCC	ATG	GAA	GGA	TTT	GGA	AAA	GGC	AAT	150
Ile	Val	Asn	Asn	His	Val	Phe	Ser	Met	Glu	Gly	Phe	Gly	Lys	Gly	Asn	
		25				30					35					
GTA	TTA	TTT	GGA	AAC	CAA	TTG	ATG	CAA	ATC	CGG	GTT	ACA	AAG	GGA	GGT	198
Val	Leu	Phe	Gly	Asn	Gln	Leu	Met	Gln	Ile	Arg	Val	Thr	Lys	Gly	Gly	
		40			45					50					55	
CCG	TTG	CCA	TTC	GCT	TTC	GAT	ATT	GTT	TCC	ATA	GCT	TTC	CAA	TAC	GGG	246
Pro	Leu	Pro	Phe	Ala	Phe	Asp	Ile	Val	Ser	Ile	Ala	Phe	Gln	Tyr	Gly	
				60					65					70		
AAT	CGC	ACT	TTC	ACG	AAA	TAC	CCA	GAC	GAC	ATT	GCG	GAC	TAC	TTT	GTT	294
Asn	Arg	Thr	Phe	Thr	Lys	Tyr	Pro	Asp	Asp	Ile	Ala	Asp	Tyr	Phe	Val	
			75					80					85			
CAA	TCA	TTC	CCG	GCT	GGA	TTT	TTC	TAC	GAA	AGA	AAT	CTA	CGC	TTT	GAA	342
Gln	Ser	Phe	Pro	Ala	Gly	Phe	Phe	Tyr	Glu	Arg	Asn	Leu	Arg	Phe	Glu	
		90					95					100				
GAT	GGC	GCC	ATT	GTT	GAC	ATT	CGT	TCA	GAT	ATA	AGT	TTA	GAA	GAT	GAT	390
Asp	Gly	Ala	Ile	Val	Asp	Ile	Arg	Ser	Asp	Ile	Ser	Leu	Glu	Asp	Asp	
	105				110						115					
AAG	TTC	CAC	TAC	AAA	GTG	GAG	TAT	AGA	GGC	AAC	GGT	TTC	CCT	AGT	AAC	438
Lys	Phe	His	Tyr	Lys	Val	Glu	Tyr	Arg	Gly	Asn	Gly	Phe	Pro	Ser	Asn	
	120				125					130					135	
GGA	CCC	GTG	ATG	CAA	AAA	GCC	ATC	CTC	GGC	ATG	GAG	CCA	TCG	TTT	GAG	486
Gly	Pro	Val	Met	Gln	Lys	Ala	Ile	Leu	Gly	Met	Glu	Pro	Ser	Phe	Glu	
				140					145					150		
GTG	GTC	TAC	ATG	AAC	AGC	GGC	GTT	CTG	GTG	GGC	GAA	GTA	GAT	CTC	GTT	534
Val	Val	Tyr	Met	Asn	Ser	Gly	Val	Leu	Val	Gly	Glu	Val	Asp	Leu	Val	
			155					160					165			
TAC	AAA	CTC	GAG	TCA	GGG	AAC	TAT	TAC	TCG	TGC	CAC	ATG	AAA	ACG	TTT	582
Tyr	Lys	Leu	Glu	Ser	Gly	Asn	Tyr	Tyr	Ser	Cys	His	Met	Lys	Thr	Phe	
		170					175					180				
TAC	AGA	TCC	AAA	GGT	GGA	GTG	AAA	GAA	TTC	CCG	GAA	TAT	CAC	TTT	ATC	630
Tyr	Arg	Ser	Lys	Gly	Gly	Val	Lys	Glu	Phe	Pro	Glu	Tyr	His	Phe	Ile	
	185					190					195					

Figure 3A
(cont.)

[illegible]

(cont.) Figure 3A

Figure 3B (cont.)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

															GACAAA	ATG	AAC	CGC	AAC	GTA	TTA	AAG	27
															Met	Asn	Arg	Asn	Val	Leu	Lys		
															1				5				
AAC	ACT	GGA	CTG	AAA	GAG	ATT	ATG	TCG	GCA	AAA	GCT	AGC	GTT	GAA	GGA	75							
Asn	Thr	Gly	Leu	Lys	Glu	Ile	Met	Ser	Ala	Lys	Ala	Ser	Val	Glu	Gly								
															10				20				
ATC	GTG	AAC	AAT	CAC	GTT	TTT	TCC	ATG	GAA	GGA	TTT	GGA	AAA	GGC	AAT	123							
Ile	Val	Asn	Asn	His	Val	Phe	Ser	Met	Glu	Gly	Phe	Gly	Lys	Gly	Asn								
															25				35				
GTA	TTA	TTT	GGA	AAC	CAA	TTG	ATG	CAA	ATC	CGG	GTT	ACA	AAG	GGA	GGT	171							
Val	Leu	Phe	Gly	Asn	Gln	Leu	Met	Gln	Ile	Arg	Val	Thr	Lys	Gly	Gly								
															40				50		55		
CCG	TTG	CCA	TTC	GCT	TTC	GAC	ATT	GTT	TCC	ATA	GCT	TTC	CAA	TAC	GGG	219							

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Pro	Leu	Pro	Phe	Ala	Phe	Asp	Ile	Val	Ser	Ile	Ala	Phe	Gln	Tyr	Gly	
				60					65					70		
AAT	CGC	ACT	TTC	ACG	AAA	TAC	CCA	GAC	GAC	ATT	GCG	GAC	TAC	TTT	GTT	267
Asn	Arg	Thr	Phe	Thr	Lys	Tyr	Pro	Asp	Asp	Ile	Ala	Asp	Tyr	Phe	Val	
			75					80					85			
CAA	TCA	TTT	CCG	GCT	GGA	TTT	TTC	TAC	GAA	AGA	AAT	CTA	CGC	TTT	GAA	315
Gln	Ser	Phe	Pro	Ala	Gly	Phe	Ile	Tyr	Glu	Arg	Asn	Leu	Arg	Phe	Glu	
		90					95					100				
GAT	GGC	GCC	ATT	GTT	GAC	ATT	CGT	TCA	GAT	ATA	AGT	TTA	GAA	GAT	GAT	363
Asp	Gly	Ala	Ile	Val	Asp	Ile	Arg	Ser	Asp	Ile	Ser	Leu	Glu	Asp	Asp	
	105					110					115					
AAG	TTC	CAC	TAC	AAA	GTG	GAG	TAT	AGA	GGC	AAC	GGT	TTC	CCT	AGT	AAC	411
Lys	Phe	His	Tyr	Lys	Val	Glu	Tyr	Arg	Gly	Asn	Gly	Phe	Pro	Ser	Asn	
	120				125					130					135	
GGA	CCC	GTG	ATG	CAA	AAA	GCC	ATC	CTC	GGC	ATG	GAG	CCA	TCG	TTT	GAG	459
Gly	Pro	Val	Met	Gln	Lys	Ala	Ile	Leu	Gly	Met	Glu	Pro	Ser	Phe	Glu	
				140					145					150		
GTG	GTC	TAC	ATG	AAC	AGC	GGC	GTT	CTG	GTG	GGC	GAA	GTA	GAT	CTC	GTT	507
Val	Val	Tyr	Met	Asn	Ser	Gly	Val	Leu	Val	Gly	Glu	Val	Asp	Leu	Val	
			155				160						165			
TAC	AAA	CTC	GAG	TCA	GGG	AAC	TAT	TAC	TCG	TGC	CAC	ATG	AAA	ACG	TTT	555
Tyr	Lys	Leu	Glu	Ser	Gly	Asn	Tyr	Tyr	Ser	Cys	His	Met	Lys	Thr	Phe	
		170				175						180				
TAC	AGA	TCC	AAA	GGT	GGA	GTG	AAA	GAA	TTC	CCG	GAA	TAT	CAC	TTT	ATC	603
Tyr	Arg	Ser	Lys	Gly	Gly	Val	Lys	Glu	Phe	Pro	Glu	Tyr	His	Phe	Ile	
	185					190					195					
CAT	CAT	CGT	CTG	GAG	AAA	ACC	TAC	GTG	GAA	GAA	GGA	AGC	TTC	GTG	GAA	651
His	His	Arg	Leu	Glu	Lys	Thr	Tyr	Val	Glu	Glu	Gly	Ser	Phe	Val	Glu	
	200				205					210					215	
CAA	CAC	GAG	ACG	GCC	ATT	GCA	CAA	CTG	ACC	ACA	ATT	GGA	AAA	CCT	CTG	699
Gln	His	Glu	Thr	Ala	Ile	Ala	Gln	Leu	Thr	Thr	Ile	Gly	Lys	Pro	Leu	
				220					225					230		
GGC	TCC	CTT	CAT	GAA	TGG	GTG	TAG	AAAATGACCA	ATATACTGGG	GAAAATCACC						753
Gly	Ser	Leu	His	Glu	Trp	Val										
AATATACTGG	GGAAAATGAC	CAATTTACTG	GGGAAAATGA	CCAATATACT	GTAGAAAATC											813
ACCAATATAC	TGGGGGAAAAT	GACCAATTTA	CTGGGGGAAAT	GACCAATTTA	CTGTAGAAAA											873
TCACCAATAT	ACTGTGGAAA	ATGACCAAAA	TACTGTAGAA	ATGTTACAC	TGGGTTGATA											933
ACCGTTTCGA	TAACCGTTTG	GAAGCTTG	TATACAAGTT	ATTTGGGGTC	ATTTTGTAAT											993
GTGTATGTGT	GTTGTATGAT	CTATAGACGT	CGTCATTCAT	AGCTTGAATC	CTTCAGCAAA											1053
AGAAACCTCG	AAGCATATTG	AAACCTCGAC	GGAGAGCATA	AAGAGACCGC	ACGTACACAA											1113
ATTATAATAC	CAGCAGTTGG	AATCTTTAAA	CCGATCAAAA	CTATTAATAT	ATATATACAC											1173
CCTGTATAAC	ATATATATAT	ATATATATCT	ACATAGTTTG	ATATTGATTA	AATCTGTTCT											1233
TGATCACTAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAA												1279

(cont.) Figure 3B